SNAKE VENOMS.

BEING

REPRINTS OF PAPERS CONTRIBUTED TO MEDICAL MAGAZINES.

BY

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UNDER SEC... 31 JUL 01

AN ACCOUNT OF SOME RESEARCHES INTO THE NATURE AND ACTION OF SNAKE VENOM.

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[THE work dealt with in the following pages was carried out with the aid of a grant most generously made to the writer for the purpose by the Madras Government, and it seems fitting that this record should be prefaced with an acknowledgment of the author's indebtedness to that Government for the large-hearted policy which has enabled him to carry out these researches; he is all too conscious of how little has been done in return for their generosity.]

I.—PRELIMINARY REMARKS ON THE METHODS EMPLOYED.

Method of Collecting and Storing Venom, Bile, etc.

In order to collect and store venom, bile, etc., the snake is first chloroformed and nailed on to a deal table; the blood is then collected by manipulations to be described later on, and the gall bladder is removed by making a long median ventral incision over it, cutting out the bladder together with the surrounding fat, and then making an incision into the most dependent part of the sac with a sharp pair of scissors; the bile flows into an evaporating dish, which is at once removed to a water bath kept at a temperature of 100° F.; as soon as the contents of this dish have dried the vessel is placed under the receiver of an air-pump, over concentrated sulphuric acid, and the air is exhausted; after twelve hours in the receiver the dish is removed, and the now dry bile is easily separated from the bottom of the dish, with the aid of a spatula or knife; it is at once placed in a dry glass tube, and tightly corked till required for use.

After removing the gall bladder the poison sacs are next dissected out, and their contents squeezed into a dry watch-glass, which is placed in a cupboard for a few hours till the poison dries into scales; if the atmosphere is damp, as is the case in the monsoon, the venom is dried by floating the watch-glass on a water bath at 100° F. for a couple of hours. The ease with which the poison separates from the glass when dry is remarkable; tapping the bottom of the glass is enough to separate the whole amount of venom from it in a few seconds. The dry poison is removed without delay into glass tubes previously dried at a high temperature, and allowed to cool before use; the tube is tightly corked as soon as filled. When undertaking a series, or a number of series of experi-

ments, the total stock of poison which it is intended to use is transferred to a glass mortar, finely pounded, and intimately mixed; it is then stored as before in a well-corked bottle and kept in the dark.

Every manipulation is carried on with the strictest attention to the prevention of the introduction of septic matter, all vessels being sterilised by heat beforehand, carbolic lotion 1-20 being used for such instruments as cannot be safely made hot; all corks used undergo a prolonged baking on a tin plate above the flame of a spirit lamp.

I have had frequent occasion to notice the correctness of a statement, made to me by Professor Fraser, that the poison of the Russel viper dries in long needle-shaped crystalline-looking masses unlike the shorter fracture of the dried poison of the cobra.

While working with live snakes a tourniquet of india-rubber tubing, a sharp knife, a cautery of some handy form, and a stock of crystals of potassium permanganate should always be kept handy. It is well, when possible, to have another medical man present; accidents occur when least expected, and the means to combat them should be ready at moment's notice. I should be wanting in gratitude if I failed here to acknowledge the debt I owe to Captain Samman, R.A.M.C., whose prompt action saved me from the very unpleasant consequences which might have followed a serious accident with which I met whilst handling a Russel viper. Captain Samman at once applied a firm ligature, and followed the fang puncture down to the bone with a knife; he then sucked the wound dry, and finally filled it with crystals of potassium permanganate. The result was that I escaped with nothing more than a painful sloughing wound. In the future a stock of Calmette's antivenene will always be kept at hand.

Standardisation of Solution and Mode of Administration.

When it is intended to perform a series of experiments, a rough calculation is first made of the total quantity of poison likely to be required, and a quantity in excess of this is carefully weighed out in decimals of a gram, on a scale showing $\frac{1}{10}$ mg. In order to minimise any possible error in weighing the writer never weighs out less than 20 mg. of poison at a time; each milligramme of venom is then dissolved in 1 or in 10 c.cm. of freshly boiled and cooled water, according to the strength of solution required. It will be noticed that these two solutions are respectively of the strength of 0.001 gram and 0.0001 gram of venom per c.cm. of water; the weaker solution is only used when the doses to be given are so small as to render their calculation difficult otherwise.

While conducting a series of experiments the solution is frequently stirred with a glass rod in order to ensure the evenness of the strength of solution. For each dose some solution is drawn up into a 10 c.cm. syringe, and the air having been expelled, the exact amount which it is required to inject is marked off by the aid of the revolving button on the piston axis; the dose is then given, either subcutaneously, or otherwise as may be desired. The syringe is sterilised by filling it several times in succession before operation from a beaker of rapidly boiling water.

Parallel series of experiments were conducted to ascertain whether the degree of dilution of the venom in the above-

named two strengths influenced the lethal properties of the dose. The length of this article forbids the publication of these tables here; but the result showed most clearly that the influence of the 10-fold dilution was inappreciable. It will be noticed, however, that in the various series of experiments one or other strength has been rigidly adhered to.

Preparation of Animal.

The animal is prepared for the injection by cutting off fur or feathers, and carefully washing the skin with 1-20 carbolic lotion.

Before undertaking any series of experiments it is necessary that all preliminaries should be carefully arranged beforehand in order to avoid confusion. Each animal of the series is therefore first weighed in pounds, ounces, and quarter-ounces; the result is converted into kilogrammes.

Means for subsequent identification are next taken; rabbits are branded in one ear with a serial number, care being taken to brand deep enough to destroy the fur and fur-bearing skin and no more; if less is done the mark wears out, while if more is done the result is sloughing, cicatrisation, and confusion; the animals scarcely seem to feel the branding. Fowls are marked by means of a wooden tally tied on under one wing. In all cases the colour, sex, etc., of the animal is noted.

The weight in kilos. being known, and the dose per kilo. having been decided on, a simple multiplication gives us the total dose the animal is to be given; and knowing the strength of our solution of venom, another easy calculation gives us the actual dose required in cubic centimetres and fractions of a centimetre.

The following columns in the experiment book can now be entered:

Serial No. of Experiment.	Date.	Hour.	Identifying Marks.	Weight in Kilos.	Dose per Kilo.	Actual Dose in c.cm.	Remarks.	Course of Events.

The first seven columns should be entered before starting to work on the series, the others subsequently.

The preparation of the mongoose requires a separate paragraph. These animals are so vicious, so wonderfully agile in their movements, and so strong and active, that it is advisable to weigh them, calculate their dose of poison, and administer it at one and the same time; they were brought in cages by the men of a caste who spend their lives in such occupations; in order to catch them, a running noose was passed through a hole in an iron rod, and this was insinuated over the animal's neck through the slightly opened door of the cage, the noose was then quickly pulled tight, and the animal was dragged out, securely tied, weighed and dealt with at once. In spite of every precaution, both I and one of my assistants received severe bites; these were delivered down to the bone in the fraction of a second, and were extremely painful.

Calculation of Dose: Idiosyncrasy of Animals.

Before starting to work on any species of animal, a long series of experiments was first undertaken to ascertain the dose of the poison to be used which would be certainly fatal in a limited period of time. At first an effort was made to work with the minimum lethal dose per kilo., but it was soon

found that this method introduced many difficulties. It was found, for example, that a dose of cobra venom corresponding to 0.0004 gram was the lowest dose per kilo. fatal to a rabbit; on the other hand, higher doses than this from time to time proved non-lethal, and rabbits occasionally recovered from doses as high as 0.0007 per kilo., though this was very rare.

If one fact stood out in bolder relief than any other it was this: that the personal element (if one may be excused the term applied to animals) is a factor never to be forgotten. Granted that the greatest care is taken to weigh the animals under the same conditions, that the poison is from the same stock, and most carefully weighed and apportioned, that every effort is made to inject the venom into the same tissues in all experiments, and to keep all known conditions as even as possible, granted all this, there still remains an element of uncertainty as to the fate of the subject which I can only attribute to "the personal factor." In order to eliminate this factor as far as possible, the dose chosen has always been one capable of certainly causing death, or at least of producing serious symptoms of snake poisoning.

In order to impose a further check on results, no series of experiments was undertaken without putting aside one or more animals as control snake-poison subjects. By this means the standardising of the lethal dose was kept as accurate as possible. This was the more necessary, because it was noticed that certain batches of animals presented a greater resistance than others, possibly due to the method of feeding, etc.

Lethal Dose for Various Animals.

Rabbits will often appear to get over the first effects of the venom, and will look as if on the way to recovery, but loss of weight continues, and after a longer or shorter period animals will succumb which at first sight one hoped would survive; their lower resistance to these secondary effects of venom poisoning renders them very inferior to fowls as subjects; the latter make a better fight to begin with, and do not suffer to anything like the same extent from remote sequelæ. Rabbits in the hills of India are also very susceptible to the influences of the frequent and trying changes of climate which prevail.

Rabbits.

All doses of cobra venom below, and including 0.0003 gram per kilo. of body weight proved non-lethal; doses of 0.0004, 0.0005, and 0.0006 gram per kilo. were lethal in most cases, but could not be certainly relied on; even 0.0007 gram per kilo. occasionally failed to kill, but it never failed to produce grave and well-marked signs of cobraism; doses of 0.0008 and 0.0009 were fatal in a very few hours. A dose of 0.0007 gram per kilo. killed in from six to eighteen hours as a rule, but life might be prolonged for days; it was accordingly selected as the dose for most of the experiments. The above conclusions appear to harmonise well with Professor Calmette's observations on dosage in his lecture delivered in the laboratories of the Royal Colleges on July 27th, 1896.

Daboia venom in doses of 0.0004 up to 0.0009 took a fortnight or more to kill; even so high a dose as 0.001 took twelve days to kill, and one of 0.002 took seven days; a dose of 0.003, however, killed with very fair uniformity in under twelve hours; 0.004 killed in four hours and a-half. The dose of 0.003 gram per kilo. was, therefore, chosen for daboia poison experiments.

Fowls.

Cobra venom in doses up to and under 0.001 proved non-lethal; 0.0015 gram per kilo. killed in fifty hours; 0.002 and 0.0025 killed in over twelve hours, and 0.003 in between six and twelve hours; these latter doses were therefore selected.

Daboia venom in doses of and above 0.003 gram per kilo. of body weight proved fatal; 0.003 gram killed in about twelve hours; all higher doses also proved lethal.

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II.

ON THE SOURCE OF IMMUNITY WHEN COBRA VENOM IS TAKEN BY THE MOUTH.

IN a paper published in the BRITISH MEDICAL JOURNAL of July 17th, 1897, Professor Fraser wrote as follows:

> The failure of so highly toxic a substance (serpents' venom) to produce poisoning when it is administered by the stomach might be due to chemical changes produced upon it by the secretions of the stomach and intestines, or to non-absorbability into the blood from the stomach and intestines. It is already known that the toxicity of venom is not reduced materially by gastric digestion......As serpents' venom introduced into the stomach is not rendered innocuous by the stomach secretions, while notwithstanding it fails to cause poisoning, it may be assumed that the stomach walls are incapable of absorbing it also. If, like other poisons, it can be absorbed from the intestines, the explanation of the failure to produce toxic symptoms when it is administered by the stomach might depend on a chemical or physiological destruction of its toxic properties by some substance or substances which it encounters soon after entering the intestinal canal, and most probably, therefore, by the bile or the pancreatic secretion. To explain the innocuousness of stomach administration fully would accordingly require that the effects on venom of the biliary and other intestinal secretions should be investigated, and also the absorbability of venom through the intestinal walls.
>
> As a contribution to the settlement of the question I have made a number of experiments with the biliary secretion, and whatever may be the influence of the other secretions, or of intestinal absorption, that of the bile has been found to be so decided as to be in itself sufficient to account for the innocuousness of stomach administration.

The object of the present paper is to fill in some of the gaps suggested by Professor Fraser in the above very interesting sentences.

Another possibility occurred to me—namely, that the source of protection might perhaps lie in the action of intestinal epithelium during absorption. This idea was suggested by the fact that the epithelium of the serpents' venom glands has the power of so altering the fluid passing through it as to change it from an innocuous fluid into a highly poisonous one, might not the action of intestinal epithelium during absorption be to invert this process?

In order to test the truth of this suggestion it was necessary experimentally to so introduce venom into the alimentary canal as to exclude the action of the various juices on the poison. With this end in view a series of experiments was undertaken which will be dealt with *seriatim*. It will be found that another explanation has been forthcoming, and that, therefore, the theory above stated has had to be abandoned. It is only mentioned as it formed the starting point of the series of experiments which are about to be described.

Feeding an animal with cobra venom after excluding the bilary secretion from the alimentary canal.

Two experiments were performed on dogs, thus: The abdomen was opened, the common bile duct securely tied, and an artificial biliary cutaneous fistula established; the intestines were swept clear of bile by purgatives, and the dog was so controlled that it could not reach the escaping bile with its mouth; a large dose of cobra venom was then given by the mouth, in one case in the dry state on meat, and in the other dissolved in milk; in both cases the dose of venom was repeated to make security secure; neither of the animals showed any sign of being the worse for the venom (*vide* Appendix A).

In view of the above facts it was obvious that, assuming the venom to have been absorbed, we must look farther than the action of bile for the immunity exhibited by the animals. It is obviously necessary to dispose of this assumption first. Later on in this paper I shall deal with the question of the influence on certain other animals of the ingestion of cobra venom by the mouth, and I hope there to show good cause to believe that venom taken by the mouth produces a marked constitutional effect upon the animal; this fact in itself would dispose of the question of absorption of venom through the intestinal walls, but more direct proof is not wanting. A reference to Appendix A, Experiment II, will show that the small gut was empty throughout twenty-four hours after the administration of the milk; the stomach was also empty. Final and convincing proof is, however, obtained when we come to the result of Experiment I of Appendix B; here we find that the venom introduced dissolved in milk fifty-five minutes earlier through an artificial opening in the small gut, had been so rapidly absorbed as to cause death in this short time; that the venom had been absorbed through the small gut was evident, not merely by its lethal effect, but also by the local changes produced, which were strictly comparable to those seen when venom is injected subcutaneously; there was, in fact, a gelatinous swelling of the intestinal and submucous membranes which closely resembled the gelatinous swelling we are familiar with in the subcutaneous tissues after injection of poison there. This absorption, be it noted, took place through an unbroken epithelial surface.

Having thus disposed of the question of the power of the intestine to absorb cobra venom, we may pass on to the next series of experiments.

The injection of cobra venom through an artificial opening in the small gut, the conditions being so arranged that the contents of the gut above the artificial anus passed out through that orifice, the peptic, pancreatic, and biliary secretions being thus shut off from the lower segment of gut, into which the venom was injected.

The above conditions were fulfilled by drawing out a loop of small gut through a 2-inch abdominal incision, fastening this gut in position, and later establishing an artificial anus in it. Time was allowed for the parts to heal soundly, and then the cobra venom was injected into the lower segment of gut dissolved in milk (*vide* Appendix B).

A reference to Experiment I of Appendix B will show that the venom, under these conditions, was rapidly absorbed and powerfully lethal. It will be noted that in this experiment the dose was but little in excess of that which the two dogs dealt with in the experiment of Appendix A took with impunity; a reference to Experiment II of Appendix B will show that one-tenth of this dose injected into the bowel did not produce any marked symptoms, while half this large dose given some two days later killed the animal in an hour and a-quarter. It is to be borne in mind that even the small dose, which when injected into the small gut proved ineffective, would have been speedily fatal had it been given subcutaneously.

From the above facts we may draw two conclusions—namely:

1. That cobra venom can be absorbed through the walls of the small gut, but not so readily as through the channels of subcutaneous tissue.

2. That some change or changes are wrought in swallowed cobra venom before it has time to reach a rapidly absorbing area.

Professor Fraser has shown in the very valuable paper I have already referred to that the bile is capable of protecting the organism from the toxic action of serpents' venom. I have repeated many of Professor Fraser's experiments, and have satisfied myself of the accuracy of his conclusions as to the antidotal powers of bile. The experiments of Appendix A point, however, clearly to the fact that bile is not the only protective agent. In the remarks already quoted Professor Fraser suggested that pancreatic juice might digest and neutralise serpents' venom. I have tested this and found that trypsin is capable of reducing very materially the lethal properties of cobra venom when the two are kept at a temperature of 100° F. over a water bath in an alkaline solution. A summary of the experiments which have led up to this conclusion will not be out of place.

First Series (October 26th, 1899).—Four rabbits were used, one being a control and the other three receiving respectively venom digested with pepsin in 0.2 per cent. solution of hydrochloric acid, venom digested with trypsin and sodium carbonate, and venom mixed with 0.007 gram of cobra bile. In the whole series 0.0007 gram of cobra venom per kilo. of the rabbit's body weight was used. The indications were not conclusive, but it appeared (1) that the pepsin had had no action on the venom, (2) that too much sodium carbonate had been given, and (3) that the bile had acted protectively.

Second Series (October 28th, 1899).—This series practically confirmed the above conclusions. They were performed before the results of the former series had had time to be known, and therefore one was unable to profit by the experience the results of the first set ultimately brought.

Third Series (November 16th, 1899).—In this series the dose was raised to 0.0008 gram of cobra venom per kilo. of the rabbit's body weight. The bile experiment was omitted as it had been more exhaustively dealt with elsewhere, and an experiment was added to ascertain whether it was likely that the absorption of the products of digestion of proteids in any amount likely to be produced by the action of the digestive ferment or venom would in itself be fatal. The control venom animal died in 6 hours and 50 minutes; the pepsin experiment animal died in $7\frac{1}{2}$ hours, while the trypsin experiment animal suffered but little at the time and survived 20 days; here again it seemed probable that the amount of sodium salt used was

too large. In any case, however, a distinct protection had been established. The result of the control experiment on the injection of peptones was death 12 days after injection, but it is to be remembered that 25 times as much egg albumen was used for this experiment as poison in the remaining experiments, and that therefore the products of digestion produced were in great excess of what can have been produced in the venom experiments.

In all these experiments the solution of venom was at a strength of 0.0001 gram per c.cm. of water, and digestion was carried on for two hours at a temperature of 100° F., the correct degree of warmth being maintained by means of a water bath.

The next series of experiments it appears worth while to give *in extenso* in Table I.:

TABLE I.—*Experiments directed to Ascertain whether Pancreatic Digestion Lessens the Lethal Properties of Cobra Venom.**

Date. (Nov., 1899.)	Weight in Kilos.	Dose per Kilo.	Remarks.
21st	1.6720	0.0007 gram venom	Control; died in 34 hours 10 minutes.
21st	1.9056	0.0007 ,, venom 0.0050 ,, trypsin 0.0050 ,, sod. carb.	There was no marked constitutional disturbance for some days. The rabbit gradually lost flesh, there was some sloughing at the seat of injection, and it died 17 days after the operation.
21st	1.9836	0.0008 ,, venom 0.0050 ,, trypsin 0.0050 ,, sod. carb.	This rabbit closely followed the same course as the previous one, but there was more local reaction. It died on the 17th day.
21st	2.0190	0.0009 ,, venom 0.0050 ,, trypsin 0.0050 ,, sod. carb.	Died in 32 hours.

* The venom (cobra) was dissolved at a strength of 0.0002 gram per c.cm. of boiled water. The solutions were kept at a temperature of 100° F. for 2 hours after the addition of the trypsin and carbonate of soda; they were then injected into the flanks of rabbits.

NOTE.—It is possible that climate had something to do with the deaths of the second and third rabbits, as they died during a very cold night. Rabbits are very susceptible to cold and damp in the hills of India, and this will obviously work at a greater advantage in lowering conditions of nutrition such as those which follow the injection of snake venom.

It will be at once noticed that the rabbit which received the large dose of 0.0009 gram per kilo. of body weight, and which under ordinary circumstances could not have been expected to live more than two to four hours, survived thirty-two hours, living nearly as long as the control which had only received 0.0007 gram per kilo.; it will also be noted that the two other rabbits which had received trypsinised venom showed a very marked degree of protection, and indeed it is doubtful whether they would have succumbed had the weather been less severe at the time. Owing to the sensitiveness of rabbits to climatic influences, and to their slight resistance to the after-effects of venom, which has been elsewhere referred to, the next series of experiments was carried out on fowls. The facts contained in Table II leave no room for doubt as to the power of trypsin to reduce the lethal properties of snake or at least of cobra venom.

Table II may be divided into three sections, each consisting of a control venom experiment, and three trypsin experiments. In the first section the dose of venom used was 0.0015 gram,

in the second 0.00175 gram, and in the third 0.002 gram per kilo. of the fowl's body weight. It had previously been ascertained by experiment that even the smallest dose mentioned was fatal in one or two days, while the highest was more rapidly fatal.

TABLE II.—*Experiments designed to ascertain whether Pancreatic Digestion Destroys the Lethal Properties of Cobra Venom.**

Date. (Dec., 1899.)	Weight in Kilos.	Dose per Kilo.	Course of Events.
16th	0.69425	0.00166 gram venom	Dead in 23 hours 40 minutes. Control.
16th	0.92090	0.00150 ,, venom 0.00033 ,, trypsin	Sick for 1 day; recovered.
16th	0.78630	0.00150 ,, venom 0.00100 ,, trypsin	Never seriously ill; recovered.
16th	1.00590	0.00150 ,, venom 0.00300 ,, trypsin	,, ,, ,,
15th	0.80760	0.00175 ,, venom	Died in 60 hours. Control.
16th	1.26100	0.00175 ,, venom 0.00033 ,, trypsin	Sick for 1 day; recovered.
16th	0.86427	0.00175 ,, venom 0.00100 ,, trypsin	Never seriously ill; recovered.
16th	1.02000	0.00175 ,, venom 0.00300 ,, trypsin	,, ,, ,,
16th	0.80050	0.00200 ,, venom	Died in less than 40 hours. Control.
16th	0.80760	0.00200 ,, venom 0.00033 ,, trypsin	Sick for 1 day; recovered.
16th	0.89950	0.00200 ,, venom 0.00100 ,, trypsin	Never seriously ill; recovered.
16th	0.87200	0.00200 ,, venom 0.00300 ,, trypsin	,, ,, ,,

* The venom was dissolved in freshly-boiled cooled water at a strength of 0.001 gram of venom per c.cm. of water. In all but the control experiments the solution was made just alkaline by the addition of sodium carbonate, and the trypsin was then added (Merck's trypsin). All the solutions alike were then placed in a water bath at 100° F., and kept there for 3 hours. At the end of that time, they were removed and injected subcutaneously in the subcutaneous tissue under the wings of the fowls.

NOTE.—In the first experiment it was intended to give 0.0015 gram of venom per kilo as a control, but, owing to a mistake, 0.00166 gram was given.

A well-marked instance of what has above been called the "personal factor" is to be observed in the order in which these control fowls died; this does not, however, affect the deductions from the series of experiments since all the control fowls did die, while of those which received trypsinised venom every one recovered; it would appear that the smallest dose of trypsin used was rather low, for the fowls which received poison treated with this low dose of the ferment were sick in every case at first. All the fowls alike showed the usual loss of weight, so they were presumably somewhat, though not severely, affected by the venom.

This closes the case for the action of the ferment of pancreatic juice on the lethal properties of cobra venom.

Taking into account that absorption of venom is far less rapid from the gut than from the subcutaneous or muscular tissues, the above shown properties of trypsin will readily

explain why venom fails to kill when swallowed, even after the protective influence of bile has been excluded, while it kills certainly, if in large enough quantity, when both the biliary and pancreatic secretions are excluded from the area of intestine which has to deal with the venom.

There are one or two other interesting facts which may be mentioned in this place. The first is that I have been unable to produce symptoms or signs of cobraism by injecting into the rectum of a dog even large doses of cobra venom; as much as 0.5 gram was used for one injection on a 10-kilog. dog, which was carefully watched to make sure the venom was not returned; it had been given dissolved in milk and water.

Another point of interest was the readiness with which venom was taken from the peritoneal cavity; it is a very easy matter to inject a solution of venom into this cavity in rabbits, as the abdominal walls are very thin, and the needle can be felt passing into the sac by the sudden lessening of resistance to its progress; it can then be freely moved in the cavity; it was found that a dose of cobra venom which would take about seven hours to kill when injected subcutaneously, would prove lethal in less than three hours when it was delivered into the abdominal cavity; a farther point of interest was that in all intraperitoneal injections symptoms of poisoning commenced much earlier than in the case of subcutaneous injections of a similar dose of venom.

TABLE III.—*Experiments directed to Ascertain whether Feeding Rabbits on Cobra Venom affords Protection against the Effects of the same kind of Venom subsequently Injected Subcutaneously.**

Serial No.	Date.	Weight in Kilos.	Dose per Kilo.	Result.
I	27/9/99	1.559	0.1240 gram by mouth	Died in 2 hours 50 minutes. *Post-mortem* examination showed that the injection had been made into the peritoneal cavity. This explained the rapid death.
	2/10/99		0.0210 ,, ,, ,,	
	6/10/99		0.0416 ,, ,, ,,	
	12/10/99		0.0330 ,, ,, ,,	
	13/10/99		0.5000 ,, ,, ,,	
	14/10/99		0.0007 ,, injected into the flank	
III	14/10/99	1.2042	0.0140 ,, by mouth	Died in 6 hours 14 minutes after the injection.
	15/10/99		0.0154 ,, ,, ,,	
	16/10/99		0.0700 ,, ,, ,,	
	17/10/99		0.0700 ,, ,, ,,	
	18/10/99		0.0890 ,, ,, ,,	
	19/10/99		0.7000 ,, ,, ,,	
	21/10/99		0.0007 ,, subcutaneously	
XI	21/10/99	1.3390	0.0016 gram by mouth	1/11/99. Sickened soon after injection; at 10 P.M. was breathing jerkily and with difficulty. 2/11/99. Lying prone and nearly paralysed at 8 A.M.; died at 10.10 A.M.—that is, 19 hours 40 minutes after injection.
	25/10/99		0.0070 ,, ,, ,,	
	26/10/99		0.0140 ,, ,, ,,	
	27/10/99		0.0560 ,, ,, ,,	
	28/10/99		0.0700 ,, ,, ,,	
	29/10/99		0.0700 ,, ,, ,,	
	30/10/99		0.1400 ,, ,, ,,	
	31/10/99		0.7000 ,, ,, ,,	
	1/11/99	1.530	0.0007 ,, subcutaneously	

* The poison in all these experiments was dissolved in a small quantity of water and then mixed with chopped cabbage leaves; the animals ate the mixture readily.

The Effects of the Ingestion of Cobra Venom in a Healthy Animal.

Professor Fraser, writing in the BRITISH MEDICAL JOURNAL of April 18th, 1896, pages 958-959, gives the result of certain experiments he conducted, which led him to the conclusions summarised in the following words:

The extraordinary result was thus obtained that serpents' venom introduced into the stomach in large quantity—in a quantity which, if injected under the skin, would be sufficient to kill 1,000 animals of the same species and weight—while it failed to produce any definite symptoms of poisoning, nevertheless produced *in a few hours* complete protection against the lethal effect of a dose of venom more than sufficient to kill the animal. (The italics are not in the original.)

The BRITISH MEDICAL JOURNAL of August 8th, 1896, page 346, in summarising Professor Calmette's valuable address delivered at Carlisle, makes the following noteworthy statement: "*He* (Professor Calmette) *had never obtained immunity by feeding animals on venom.*" (Again the italics are not in the original.)

I accordingly instituted a number of experiments to ascertain, if possible, what the cause of the discrepancy in evidence was. A review of Tables III, IV, and V will show the results obtained.

TABLE IV.—*A Controlled Series of Experiments directed to Ascertain whether Feeding Rabbits on Cobra Venom affords them Protection against the Effects of the same kind of Venom subsequently Injected Subcutaneously.**

Serial No.	Date.	Weight in Kilos.	Dose of Venom per Kilo.	Result.
LV	28/10/99	0.9070	0.0007 gram injected into flank as control	28/10/99. Not seriously unwell. 29/10/99. Unwell, but eating, and decidedly better than rabbits of next three experiments. 21/11/99. Was ill for several days, then improved, but emaciated, and died 24 days after injection.
LVII	27/10/99 28/10/99	0.9068	0.4500 gram by mouth 0.0007 ,, subcutaneously	28/10/99. Very sick to-night. 29/10/99. Very sick, not eating. 3/12/99. Was very sick for several days, then improved, but emaciated, and died on the 36th day.
LVIII	27/10/99 28/10/99	0.8501	0.7000 gram by mouth 0.0007 ,, subcutaneously	This rabbit followed much the same course as the previous one, and died on the 11th day with a sloughy wound at the seat of injection.
LIX	27/10/99 28/10/99	0.8500	0.7000 gram by mouth 0.0007 ,, subcutaneously	Died 7 hours after injection.

* The venom was administered in the same way as in the previous experiments.

NOTE.—The hypodermic injections of venom on the 28th were all made in the flank.

In the earlier experiments (Table III) no control animal was used, as it was expected that the protection would be too well marked to need control. In view of the conclusions eventually arrived at, we may neglect the first experiment, on the ground that, as the injection was made accidentally into the peritoneum, the test was unfairly severe. Experiments III and XI were free from this objection; it will be noted that a dose of venom was given nearly every day for a week in III, and for a slightly longer period in XI; the earlier doses were comparatively small, while the last dose in each case was one which, if given subcutaneously, would have been fatal to 1,000 animals of the same weight and species. There was no evidence of protection in either of these cases; on the contrary, the animals seemed to have been markedly influenced unfavourably by the swallowed venom, so far as their power of resisting the lethal effects of the venom went.

Two series of experiments were therefore undertaken; the first of these is comprised by Experiments LV-LIX (Table IV). The most noteworthy point here was that during the few days immediately following the injection the control rabbit was decidedly less ill than any of those which had beforehand received venom by the mouth. The control rabbit died in 24 days, while the other 3 died respectively in 7 hours, 11 days, and 37 days after injection. It is evident that no case for protection can here be established.

TABLE V.—*Farther Experiments on the Acquisition of Immunity by Feeding Rabbits.**

Serial No.	Date.	Weight in Kilos.	Dose per Kilo.	Remarks.	Result.
85	21/11/99	1.22	0.3500 gram	By mouth	*Nil.*
	22/11/99	1.22	0.0007 ,,	Subcutaneously	Sickened in a few hours, and was dead 24 hours 50 minutes after injection.
86	21/11/99	1.785	0.3500 ,,	By mouth	*Nil.*
	22/11/99	1.785	0.0007 ,,	Subcutaneously	Sickened rapidly, and died in 33 hours 40 minutes.
87	21/11/99	1.813	0.0007 ,,	Subcutaneously	This rabbit was less ill than either of the preceding ones, and, though sick for a few days, gradually got better. It died 24 days after the injection, greatly emaciated.

* The poison was dissolved at a strength of 0.001 gram. per c.cm. of water.

Another series of experiments was undertaken on November 21st and 22nd, and here the indication seemed more clear than ever, for the control lived 24 days, while the two rabbits, which on the previous day had each received by the mouth 500 times the subcutaneous lethal dose, died respectively in 24 hours 50 minutes and 33 hours 40 minutes.

Throughout all these experiments the writer could not help observing that though feeding an animal on snake poison did not appear at the time to affect it injuriously, yet when a dose of poison subcutaneously given was superadded, the symptoms

of cobraism were more rapidly developed and more markedly pronounced than would have been the case in an animal which had only the subcutaneous dose of poison to contend with.

From this it is surmised that cobra venom when taken by the mouth passes in some form, probably an altered one, into the general circulation. It is likely that if time is given for the disposal of these products their effect will be found to be in the direction of immunisation, but in the meantime they appear to affect the animal's resistance to the venom prejudicially.

I have noticed a very similar phenomenon with regard to sublethal doses of venom subcutaneously administered; if sufficient time is allowed to elapse between the injections, the animal acquires a protection against the influence of the venom, but if the doses are given at too short intervals, death will result from what otherwise would have been a sublethal dose. These facts appear to point to the inference that it takes the organism some little time to deal with venom, however administered, and that in the earlier stages of this complex and little-understood process the animal is more susceptible to poison than it ordinarily would be. There is no want of analogy in the action of other similar protective processes.

This is not, as it may at first sight appear to be, a question of purely theoretical interest, for on it hinges the correctness or otherwise of Professor Fraser's views as to the source of immunity among snake charmers all the world over. I thoroughly believe those views to be correct so far as Indian snake-charmers are concerned, but I am disposed to disagree with Professor Fraser as to the rapidity with which the immunity can be acquired by animals. It is always to be remembered, however, that it is a common practice of Indian snake charmers to rub the poison of cobra on to the back of one hand with the palm of the other; this habit, which one frequently sees the snakeman practise, must often lead to the absorption of venom through abrasions, even if this is not deliberately aimed at. Possibly it is to this practice that Professor Fraser refers when he speaks of "inoculation of venom" being "apparently sometimes practised" by snake charmers.

It is more than likely that the mongoose owes its large share of immunity against snake venom to its practice of devouring the head of its victim as soon as the victory has been won. I shall have more to say on this subject on a subsequent occasion when dealing with the acquired resistance of the mongoose. For the present it only remains for me to express the regret I feel in differing, even in a minor matter, from one to whom all workers in the field of snake poison research owe so much as they do to Professor Fraser.

There are many to whom I would like to express my acknowledgements did space allow, but I should be indeed remiss did I forgot to mention Lieutenant-Colonel Price, I.M.S. (retired), Captain Samman, R.A.M.C., Mr. C. Grey, and Mr. Balavendaram, all of whom have given valuable assistance in carrying out my experiments. Last, though far from least, I desire to acknowledge the kind find ready assistance given by Mr. C. H. Mounsey, Collector of Coimbatore, and Mr. C. Weir, Collector of the Nilgiri District, in obtaining snakes, animals for experimental purposes, etc.

Appendix A.

Experiments Directed to Ascertain whether the Influence of Bile is the Sole Protective Factor when Cobra Venom is administered by the Mouth to Animals.

Experiment I.

Date.

2/10/99 A black pariah bitch, weight 13.6 kilos., was chloroformed, the abdomen opened with antiseptic precautions, and the common bile duct tied close to the duodenum; the gall bladder was opened, emptied, and sutured to the peritoneum (parietal) at the upper part of the wound, a tube inserted, and the rest of the wound carefully closed, a biliary fistula being thus left; the dog recovered well from the anæsthetic.

3/10/99 No signs of peritonitis; dog doing well.

4/10/99 Dog well and brisk. At 8.13 A.M. a dose of cobra venom (dry), weighing 0.30 gram, was given by the mouth on meat, and a similar dose repeated at 11.45 A.M. The dog thus took an amount of cobra venom corresponding to a dose of about 0.044 gram per kilo. of body weight.

5/10/99 The dog has not been any the worse for the venom. No sign of cobraism appeared. A dose of castor oil was given and the dog was freely purged thereby.

6/10/99 Passed two stools during the night: the first was well bile-stained, the second quite white; bile escapes freely from wound; fresh dose of oil given.

7/10/99 The dog had got free from its bandages during the night, and had been licking the bile from the wound; a fresh dose of oil was given, and the dog was securely tied, so that it could not reach the fistula with its mouth; the dose of oil was repeated in the evening to clear out any bile that had been licked up.

8/10/99 Dog passed quite white stools this morning, and so at 7.30 A.M. she received by the mouth a dose of 0.62 gram of cobra venom, that is, a dose of 0.045 per kilo.; at 10.30 A.M. she was brisk and wagging her tail.

9/10/99 The dog has not been at all sick, and is wagging her tail; seems in excellent health.

10/10/99 The dog is in excellent health; she passed white stools last night; at 7 A.M. a dose of 0.13 gram of cobra venom (0.01 gram nearly per kilo.) was given hypodermically. The dog quickly sickened, and was dead at 3.30 P.M., that is, in eight and a half hours.

Post-mortem Examination at 6 P.M.—Following down the common duct from the external opening of the biliary fistula, it was found that the ligature had been applied to the duct beyond all branches; the contents of the intestines were tested for bile, but the result was quite negative: there was no appearance of bile staining anywhere on the surface of the intestines. The stomach was full of food.

Experiment II.

Date.

10/11/99 Under chloroform the abdomen of a pariah bitch (weight, 10 kilos.) was opened, the gall bladder drawn out, packed with sponges, and then emptied; the common bile duct was found, pulled out from the gut for the space of ½ inch, and ligatured as close to the gut as possible; the external wound was then closed, an artificial biliary fistula being established externally in the same manner as in the last operation. The gall bladder was very full, as the dog had been fasting over night. To avoid all danger of the animal licking up bile from the wound, it was so fastened that it could not reach any part of its body with the mouth.

11/11/99 Dog doing well; no motion passed.

12/11/99 7.30 A.M.—2 ounces of castor oil given by mouth. 6 P.M.—Bowels acted; the first part of the motion was slightly bile-stained, the last part quite white.

13/11/99 Bowels acted early this morning, colour quite white; 0.5 gram of cobra venom was given by the mouth dissolved in milk at 10 A.M. The dog evidently disliked the taste, and the milk was poured down its throat; all the milk was retained. The dog seemed none the worse for the dose, and was quite brisk in the evening.

18/11/99 The animal did not at any time seem at all the worse for the venom; bile continued to escape freely from the fistula, and daily motions destitute of bile were passed. At 8 A.M. another dose of 0.50 gram of cobra venom was given dissolved in milk; the poison again appeared to burn her mouth, but she was easier after taking some broth; the whole quantity of milk was retained; she made no effort to vomit any of it. On each occasion the dose of poison corresponded to 0.05 gram per kilo. of her body weight.

19/11/99 The dog was in good health, and had shown no signs whatever of cobraism; she was given an overdose of chloroform, and a *post-mortem* examination made at once.

Post-mortem Examination.—The adhesive process around the sutured gall-bladder was firm and healthy; there was no other evidence of peritonitis; the common duct was traced down from the fistulous opening in the gall-bladder, and was slit up till it joined the duodenum; the silk ligature used was found retracted into the walls of the gut, and the duct was quite impervious till the ligature was cut; the whole length of the duct was then slit up into the gut, and a very marked contrast was noticed in the colour of the mucous lining of the duct above and below the site of the ligature; above it was stained a deep brown by bile, while below the surface was white and glistening. The small gut was empty throughout, and was destitute of any trace of bile; the large gut contained fæcal masses also destitute of bile. The stomach was empty, and natural in appearance.

APPENDIX B.

Experiments to Ascertain whether Cobra Venom introduced into the Alimentary Canal of a Dog under such circumstances that the Peptic, Pancreatic, and Biliary Secretions cannot act upon it, retains its Lethal Properties.

EXPERIMENT I.

Date.

23/11/99 The abdomen of a pariah bitch was opened, under chloroform, a coil of small gut was drawn out, and fixed outside the abdominal wall by means of a silk suture passed through the whole thickness of both lips of the incision and the mesentery of the coil; the suture, which was a double one, was fastened by tying its ends over two buttons, one at either side; a small horsehair suture at each end of the incision including the peritoneal coat of the gut made all secure. The dog had been prepared for operation by a dose of oil overnight and an enema in the morning.

25/11/99 The portion of gut above the skin surface was cut away with scissors; the mesenteric or posterior wall of the gut thus projected, forming a spur which effectually shut off the upper and lower openings of the artificial anus from each other.

27/11/99 The edges of the incision in the gut have healed, and the contents of the upper segment are voided freely, and show no tendency to pass into the lower segment's artificial orifice.

4/12/99 The dog has made a good recovery, and is gaining flesh. Its weight is a little over 9 kilos. 0.5 gram of dried cobra venom was weighed out, dissolved in 1 ounce of milk and water, and injected into the lower opening in the gut through a soft rubber catheter passed in about 5 inches; the catheter was withdrawn, and the lips of the orifice were kept closed by the pressure of the finger and thumb, so that no fluid could regurgitate. Within fifteen minutes the dog sickened, and was dead in fifty-five minutes.

Post-mortem Examination.—A *post mortem* examination was performed at once. There was a healthy adhesion of the gut to the abdominal wall, and no other evidence of peritonitis; the artificial anus was found to be in the small gut, 12 inches above its junction with the large intestine; the mesenteric vessels of the area were turgid with blood, and the glands were swollen; the small gut above the artificial anus was flaccid and flattened, while that below stood out as if full of fluid. On slitting up the small gut above and below the artificial opening it was found that the mucous membrane of the lower segment was three times as thick as that of the upper. It presented an appearance closely resembling the gelatinous exudation one sees in subcutaneous tissue after the injection there of cobra venom, and the swelling was obviously

of the same origin. This swelling accounted for the turgid state of the gut before it was slit up, for nearly all the fluid injected had disappeared and the gut was empty. The poison solution had evidently been absorbed through the walls of the gut, and in so doing had set up an inflammatory process, the outcome of which was the exudation referred to. There was no infiltration of the subcutaneous tissue around the attachment of the gut to the abdominal walls.

Experiment II.

23/11/99 Experiment I of this appendix was repeated, with slight variations. The intestine was opened at the time of operation, and the gut edges united at once to the abdominal walls by horse-hair sutures; the posterior or mesenteric wall of the gut was drawn well forward into the wound, so as to separate the portion of gut above from that below the opening.

27/11/99 The cut edges of the intestine are healed. The dog has done well in every way.

6/12/99 The dog being in good health, and the artificial anus being in a healthy condition, an injection of cobra venom dissolved in milk and water was made into the lower orifice of the artificial anus. Weight of dog was just under 10 kilos. Total dose of poison given was 0.05 gram; therefore dose per kilo. was 0.005 gram. The dog showed no signs of suffering from this dose.

8/12/99 A dose of 0.25 gram of cobra venom dissolved in a few drachms of milk was administered, as before, by injection into the lower opening of the artificial anus. This represents a dose of 0.025 gram per kilo. of body weight. The dog was dead in an hour and a quarter.

Post-mortem Examination Four Hours after Death.—The opening of the artificial anus was situated 7 inches above the junction of the small gut with the large. As in the previous case a marked contrast was noticed between the thickness of the mucous membrane of the small gut above and below the artificial anus: above the membrane was natural; below it was much thickened, and was gelatinous in appearance; this gelatinous appearance also extended down some 5 or 6 inches of the first part of the large gut; the portions of both large and small gut which were affected in this way also had the mucous surface covered with a thick film of mucous exudation; the remainder of the intestine was normal in appearance.

In future communications it is proposed to deal with the protective properties of the serums of the cobra and Russel's viper, with the cause of the immunity possessed by the mongoose, with the nature of the protection conferred by bile, and with certain other topics.

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AN ACCOUNT OF SOME RESEARCHES INTO THE NATURE AND ACTION OF SNAKE VENOM.*

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III.

On Acquired Immunity against Snake Venom possessed by Indian Snake Charmers, by Mongooses, and by Certain other Animals.

In February, 1899, I was deputed by the Government of India to meet Professor Fraser in Bombay, and to confer with him on the best lines for the prosecution of snake venom research in India. The work which forms the subject of the present paper was then suggested to me by the Edinburgh pioneer of venom research, and I desire to preface my article with an acknowledgment of the timely help I received from him. The first step was to ascertain the extent of the reputed immunity enjoyed by the Indian mongoose and by certain other animals. The appended tables will show this portion of the work at length. Thanks to the courteous co-operation of the collector of Coimbatore, a number of mongooses were obtained, and several series of experiments were undertaken with a view to discover how much cobra venom per kilo. of body weight could be injected without causing death. The venom was in nearly every case dissolved at such a strength that 1 c cm. of solution contained 1 mg. of the poison. The injections were made into the animals' thighs with all antiseptic precautions. The results obtained are set forth in Tables A and B. In the former are massed together several series of experiments, conducted in the hills, and, therefore, in a climate which was colder than that to which the animals are accustomed; they were, with but few exceptions, in good health when operated on.

Table B sets forth the results of a set of experiments conducted in the plains, but the reliability of the results is marred by two factors, namely: (1) That the animals had been some time in captivity before I used them; and (2) that on my return to the hills I was obliged to take them up with me from a hot dry climate into cold driving rain; such a change is most trying to any constitution, and these little animals feel it acutely. I do not hesitate to attribute the deaths of the third and fourth animals in Table B to climatic influences, for I have little doubt that they would have survived had it been possible to leave them in their natural climate. I am also persuaded that the lower lethal doses which proved fatal to the animals in this table as compared with those in the previous one are to be explained by the fact that captivity had told on the health of the whole batch.

* This paper is in continuation of papers published in the British Medical Journal of February 10th, and May 12th, 1900.

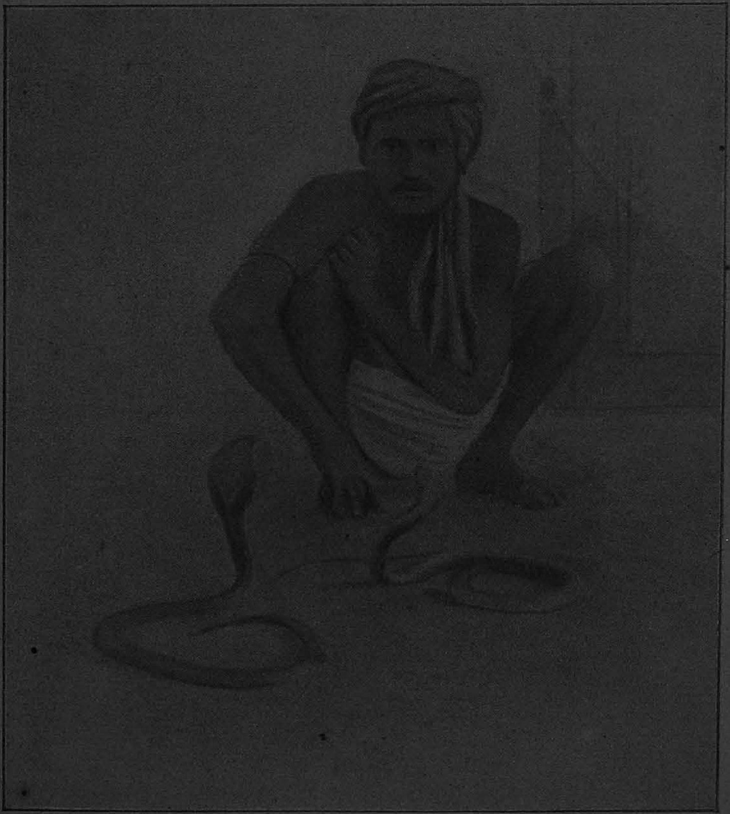
Bearing the above considerations in mind, we may now review the whole mass of experiments included in the two tables. From such a survey it would appear that a dose of 0.006 gram. of cobra venom per kilo. of body weight is likely to prove fatal to a mongoose, but that, given a healthy, active animal, much larger doses than this can be borne. It is indeed remarkable that animals should have survived doses of 0.01 gram, and 0.015 gram per kilo. of body weight. If we compare these results with those obtained by using the same stock of venom on rabbits, we find that the mongoose takes from 10 to 25 times as much cobra venom per kilo. of body weight to kill him as the rodent does. M. Calmette gives a somewhat lower estimate than mine in his most interesting work *Le Venin des Serpents.* He performed only three experiments, and found that while four times the lethal dose per kilo. of rabbit failed to make a mongoose sick, six times that dose caused two days' illness, and eight times the dose killed in twelve hours. I would particularly invite attention to the rapidity with which death ensued in this case as compared with my own results.

A very important point to remember is that the mongooses with which Professor Calmette experimented had been obtained by him from Guadeloupe, where he states that venomous snakes are unknown; they had been imported into that island 25 years before from Barbados, with a view to the destruction of the rats there

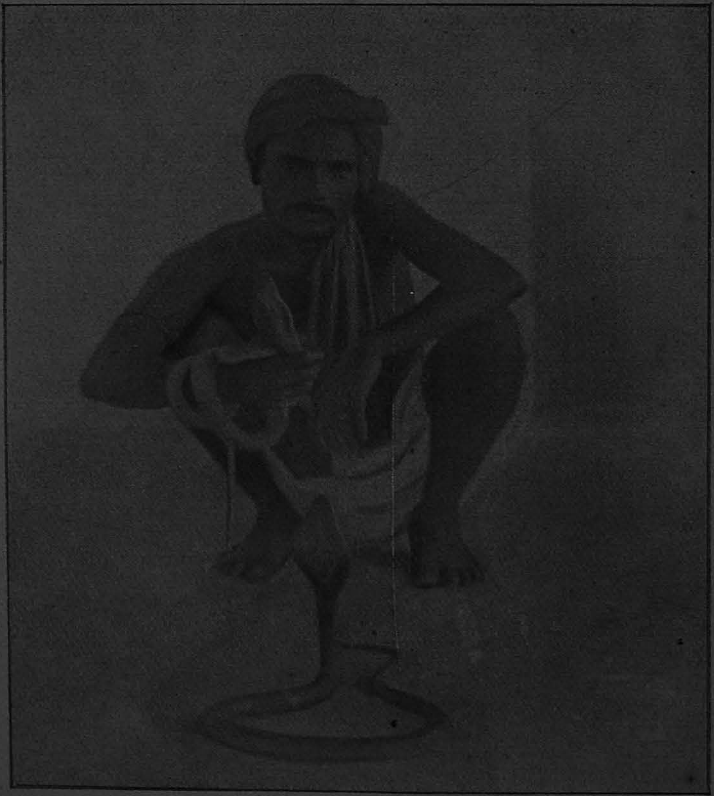
If immunity against venom is to any extent an inherited characteristic of these foes of the snake, it seems likely that the above mentioned fact has a very important and interesting bearing on the question of our relative estimates of the immunity the mongoose possesses, for my specimens came from Coimbatore (a district in the Madras Presidency), which is well known as a happy hunting ground for venomous snakes of several kinds, and especially of the cobra.

Now, the mongoose was introduced into Barbados from India subsequently to the year 1882,[1] in order that it might destroy the rats, which had proved a dangerous pest to the sugar-cane industry; assuming Professor Calmette's statement to be correct, the importation of the mongoose into Guadeloupe must have followed very close on its advent into the Barbados, so that for practical purposes we may assume that the Indian mongoose was imported direct into the former island It must now be clear that the specimens of this little carnivore, with which M. Calmette experimented, had not been away from contact with poisonous snakes much more than 25 years. We are thus led to the interesting conclusion that the introduction of the mongoose into a country in which venomous snakes are unknown has resulted, within so short a period as a quarter of a century, in a very appreciable reduction of the animal's resistance to snake venom. This fact points strongly to the further conclusion that the immunity is an acquired one, and inasmuch as the acquired characteristic has been so rapidly and easily diminished, it would appear likely that it must be maintained from generation to generation. Be it remembered that a quarter of a century probably means about 50 generations.

An important factor in discussing the protection enjoyed by the mongoose is the nature of its diet, for it is almost exclusively carnivorous in the wild state, though in captivity it will eat fruit and vegetables readily. Professor Fraser has shown reason to believe that the habitual ingestion of animal



Snake charmer at work.



Snake charmer at work.

food increases the resistance of even a herbivorous animal to snake venom; it therefore seems advisable to compare the mongoose with a carnivorous animal, and a ready type is found in the dog. According to M. Calmette, it takes 0.006 gram of cobra venom to kill a dog weighing 7 kilos. in twelve hours, while the same quantity of venom will kill 12 kilos. of rabbit in three hours; roughly, we may say that, according to this estimate, the resistance of the dog is double that of the rabbit. A reference to Table C will show my results with Indian pariah dogs; these, compared with my previously published results with rabbits, confirm the relative resistances of the two animals as laid down by the French savant. It will be noticed that, while our relative results agree, the absolute amount of venom used by me was higher than that used by Professor Calmette; this is doubtless due to my poison being from a weaker stock than his, it being well known that the strength of cobra venom varies according to the time of year in which it is collected, and according to other influences which it would be beside the point to discuss here.

Taking the rabbit as a unit, we are now in a position to draw up a table of the relative resistances to cobra venom exhibited by the dog and mongoose. We may state these roughly thus: Rabbit 1, dog about 2, mongoose from 10 to 25. It is not contended that these figures are mathematically correct, but they are believed to be as close approximations to the truth as it is possible to attain, when dealing with calculations into which that very variable factor "the personal equation" enters. One thing at least is quite clear, that we must look beyond the mongoose's food if we wish to find an adequate explanation of the source of its partial immunity to snake poison.

In order to obtain light on the subject, I have closely watched fights between wild mongooses and untamed cobras, and have also been at pains to collect reliable information on the subject from others who have witnessed similar combats. The first thing that strikes a spectator in these contests is the absolute indifference the mongoose exhibits in the presence of a snake, even after it has had one or two scrimmages with the reptile; the cobra, on the contrary, is "all there;" it raises its hood, follows every movement of the mammal, and hisses almost without interruption. As a rule, both animals evince a strong desire to avoid a fight, but there is the greatest difference between the casual conduct of the mongoose and the lively terror exhibited by the cobra. It would, however, be a great mistake to suppose that the former animal for a moment forgets the presence of a dangerous foe, for it will dart out of the way of a sudden stroke with a combined agility and *aplomb* that are astonishing. When the little carnivore finds that it cannot get away, it seems suddenly to catch sight of the reptile; it then approaches slowly and stealthily, watching every movement its adversary makes, and never allowing its eyes to leave the snake's head; now begins the "open phase" of the fight, in which the cobra's lunges are either evaded by swift backward springs, or "countered" by a sudden dash forward which results in the mongoose seizing the snake's head in its open jaws. The rapidity of this latter manœuvre is almost incredible, unless one has witnessed it.

A very noticeable point is that the mongoose always makes for the snake's head; I have never seen it attempt any other

grip, and very often one finds the jaws of the two animals interlocked. It then looks as if the mongoose must certainly be severely bitten; but that this is not the case is often shown by the result of the fight. There is something in the grip which prevents the cobra from injecting any great amount of venom. In one case I found that the snake's fangs had been snapped off short by the carnivore's teeth, and in several instances I satisfied myself that these bladed teeth had transfixed the venom sacs, doubtless giving free exit to their contents, and rendering them useless, thus putting the poison apparatus *hors de combat*. There would seem to be but little doubt that under such circumstances venom must have found its way into the mongoose's mouth and so down its throat. This is an important point to which I shall revert later on. Such a grip as I have described may be only momentarily held, in which case open sparring is resumed, or the mongoose may hold on, bull-dog fashion, till the snake succumbs. It is a gruesome sight to see the two animals rolling over and over in a merciless death grip, the snake writhing with pain while it enfolds the little mammal in its coils, the mongoose maintaining its hold with a courage that only death can damp.

There is one point I have passed over and to which I desire to return. It is the question of the frequency with which the cobra "gets home" in the earlier stages of the fight. To a casual onlooker the snake often appears to get its blow in; but a careful watch will show that as a rule it misses stroke after stroke. Even when it does succeed in striking its little foe, the agility of the latter is such that it bounds out of the way before any appreciable amount of venom can have been injected. To my mind the only chance the snake gets of injecting a full dose of poison is in the interlocking grip, when for some reason the mongoose has not succeeded in catching its adversary's head with its accustomed knack. At the same time, I do not mean to assert that the mongoose never gets a fatal bite whilst skirmishing, though I think that such an event must be uncommon.

The mongoose possesses a weapon of defence that deserves especial mention, in its long bristly hair; when attacking a cobra every hair stands on end, so that the little animal appears to be double its real size, or more; the natural result of this is that the snake is largely misled as to the true size of target available, and many a desperate lunge is wasted on the harmless "outer" of the mammal's thick coat, instead of scoring a "bull's-eye" on the lithe spare body. Many observers have laid stress on the idea that the mongoose's coat is too thick for the cobra to be able to get through it, and they have also called attention to the toughness of its skin. I attach no importance to either of these factors, which at the very most are only subsidiary items; no one who has watched the force of a cobra's lunge can seriously think them anything more; as for the skin of the mongoose it is no tougher than that of a dog, indeed it is not so tough, and one speaks after having used a hypodermic needle many times on both species of animals. The real functions of the outstanding hair are in my opinion (1) to mislead the snake as to the available size of target; and (2) to terrify its foe by means very comparable to those it itself uses in erecting its hood.

After watching a number of such fights as have been described above I feel justified in drawing the following conclusions: (*a*) That during the open phase of a fight

a mongoose is likely occasionally to receive incomplete bites, in which only very small quantities of venom are injected into its body; (*b*) that when the mammal seizes the snake's head in its mouth and drives its bladed teeth through the poison sacs the escaping poison must often pass down its throat and be swallowed; (*c*) that in both of these ways it is likely that a gradual immunising process takes place, a process which is kept up through successive generations so long as the animals are in constant contact with poisonous snakes, but whose effect dies away should the mongoose be transported to a country in which the *Thanatophidia* are distinguished by their absence. The question that will naturally be asked is, "On what evidence do you base your view that the consumption of venom by the mouth leads to the establishment of immunity or of partial immunity?" My reply to this is that though I am not prepared at the present moment to bring forward positive proof in favour of the view that some degree of immunity can be established by feeding animals on venom, a view which is associated with no less a name than Professor Fraser's, yet I hope before long to publish some evidence tending in that direction. It is a matter which requires both time and patience, as the immunity cannot be produced in a short time. It would be previous to say more at present.

That the mongoose owes its many narrow escapes to its agility as much as to its partial immunity may be gathered from the fact that many Anglo-Indians possess dogs which make a point of killing every snake they see. A case in point may be quoted: A gentleman recently retired from the Madras Railway gave me the following description of two dogs he owned for many years: "These dogs never lost a chance of attacking a snake either singly or the pair together; if they spied a snake on the verandah chicks they would whine and dance about till it was shaken down to them; they would never attack a cobra or any other snake as long as it stood up to face them, but the moment the serpent lowered its head and endeavoured to escape they would rush in, seize it by the back, and shake it so violently that the reptile had not a chance of retaliating, and was dead before one could have believed it possible; if the first grip was not a good one they would fling the snake away and again follow it up when it tried once more to escape; between them they killed very many dozens of snakes of all kinds, including many cobras; one of them at last ventured once too often, and met his death from the bite of a cobra he was worrying." Such instances are not uncommon, and they go far to prove that a quick resolute animal, unendowed with any peculiar resistance to snake venom, can hold its own with even as deadly a foe as the cobra, though the risks must always be very great.

Nor is the dog the only domestic animal which goes out of its way to attack snakes. Mr. Gray of Coonoor, an ardent naturalist and a most trustworthy and careful observer, informs me that he knew of a cat which used often to bring in specimens of the poisonous hill vipers. Another gentleman, on whose word I can rely, told me that he had a cat which used to tackle cobras; on one occasion, hearing his child shrieking with laughter, he went into a bathroom, and there discovered the cat engaging a cobra; she was sitting in front of the reptile in a boxing attitude; every time the snake lunged the cat struck the blow to one side with her paw; when the cobra made an effort to escape, and lowered its head

for the purpose, the cat seized the opportunity to rush in with a bite that brought the unfortunate reptile to the defensive again, and so on till the snake was wearied out. Other trustworthy persons have confirmed the accuracy of the above statements from their own experience.

Guineafowl and peafowl have the reputation of keeping a compound free of snakes, though with what amount of truth I am unable to say. A native who has given me much assistance in my work on snake venom assures me that he has seen a peacock kill a large cobra, and that on opening peafowl he has often found snakes in the alimentary canal. He adds that he has also seen guineafowl catch and eat young cobras or other young snakes. He is a reliable man, and he asserts that such facts are well known to natives, being matters of common experience. I cannot confirm his statements by any personal or other European evidence, but I am inclined to believe him. In this connection the following contribution from a correspondent stationed at Munzerabad is interesting: "February 7th, 1900.—Three weeks ago I saw a common grey kite attack and slaughter a Russel viper, which it afterwards carried away. The bird hovered and fluttered around, half in the air, half on the ground, striking the snake with beak and wing. I do not think the bird was touched."

TABLE A.—*Experiments Undertaken to Ascertain the Lethal Dose of Cobra Venom for the Mongoose (Herpestes Mungo).*

Serial No.	Weight in Kilos.	Dose per Kilo.	Remarks.
*VII	1.274	0.0025 gram	Never seemed unwell beyond what the cold in the hills would account for. Recovered.
*VIII	0.821	0.0050 ,,	Did not seem to feel the injection, and apparently recovered; it died four days later, 6/10/99, at 2 P.M. It was very doubtful whether the venom caused death.
*IX	0.793	0.0075	Died in 5 hours 45 minutes. This animal had been sent up from the plains in the same cage as a female and another large male, and it was noticed before injection that he had been badly bitten. The test was scarcely a fair one.
*X	0.5667	0.0100 ,,	A lively and vicious young female; never seemed any the worse for the injection.
LXIX	1.1477	0.0100 ,,	Seemed to feel it but little for the first 3 days, but died on the fifth day. Death possibly due to chill.
LXX	1.1902	0.0125 ,,	Died in 21 hours 55 minutes.
XXV	0.7467	0.0150 ,,	Recovered with but little disturbance.
LXXI	1.105	0.0150 ,,	Seemed to feel it but little the day of injection, sickened on the next, and died 31½ hours after injection.
LXXII	1.24687	0.0175 ,,	Seemed all right for some hours after the injection, but was dead in 21½ hours.
XXIV	0.65173	0.0200 ,,	Died in about 12 to 15 hours; found dead and cold in the early morning.
LXXIII	0.73677	0.0200 ,,	Did not sicken the same evening, but was dead in 23 hours.
LXXIV	0.5667	0.0225 ,,	Did not sicken that evening, but was dead in 22 hours 55 minutes.

N.B.—In the experiments marked with an asterisk (*) the poison was dissolved at such a strength that there was 0.0001 gram of poison in each c.cm. of solution. In all the other experiments the strength was such that in each c.cm. of solution there was 0.001 gram of poison.

Table B.—*A Further Series of Experiments Designed to Ascertain the Lethal Dose of Cobra Poison for the Mongoose, undertaken on November 11th, 1899.*

Weight in Kilos.	Dose per Kilo.	Remarks.
1.389	0.002 gram	Recovered.
1.8143	0.003 ,,	Recovered.
1.4174	0.004 ,,	Died on the sixth day, and 2 days after removal from the dry heat of the plains to the severe cold damp of the hills; death is attributable to this change of climate, which mongooses feel severely.
1.417	0.005 ,,	Died on the eighth day; here again there was but little doubt that death was due to the change of climate.
0.680	0.006 ,,	Died 67 hours after the injection, but had seemed quite well for the first 24 hours.
0.907	0.007 ,,	Sickened in a few hours and died in 29 hours.
0.822	0.008 ,,	Seemed well the morning after injection, but died in a little under 44 hours.
0.652	0.009 ,,	Seemed well the morning after injection, but died in 60 hours.
0.8788	0.010 ,,	Died in 40 hours.
0.8505	0.011 ,,	Sickened soon and was dead in 22 hours.
0.6237	0.012 ,,	Sickened speedily and was dead in 19¾ hours.

There is a common belief amongst natives that Indian pigs eat snakes and that they are to some extent protected from cobra bites. Lieutenant-Colonel O'Hara informs me that he has watched a sounder of wild pig trample a cobra to death with the greatest deliberation. Professor Calmette, too, evidently believed that pigs possess some measure of protection, though

Table C.—*Series of Experiments Designed to Ascertain the Lethal Dose of Cobra Venom for Pariah Dogs.*

Weight of Dog in Kilos.	Dose of Venom per Kilo.	Result.
9.5	0.00075 gr'm	Very ill, for 2 days, then slowly recovered.
14.5	0.00100 ,,	Died in 27 hours.
9.5	0.00125 ,,	Died in 7½ hours.
11.78	0.00150 ,,	Died in 4¾ hours.

Table D.—*Series of Experiments Designed to Ascertain the Lethal Dose of Cobra Venom for Indian Pigs.*

Weight in Kilos.	Dose of Venom per Kilo.	Result.
15.4	0.00075 gr'm	Seemed a little ill for 2 days and then recovered.
20.4	0.00100 ,,	Died in four days.
25.84	0.00125 ,,	Died in 40 hours.
19.72	0.00150 ,,	Died in between 12 and 17 hours.

The pigs used were the common village pigs, well known as the scavengers of Indian hamlets.

TABLE E.—*An Extract from a Series of Experiments showing the Lethal Dose of Cobra Venom for Fowls.*

Weight of Fowl in Kilos.	Dose of Venom per Kilo.	Result.
0.836	0.0010 gram	Sick for one day; recovered.
0.878	0.0015 ,,	Died in 50½ hours.
0.836	0.0020 ,,	Died in under 15 hours.

TABLE F.—*Two Experiments designed to Ascertain the Lethal Dose of Cobra Venom for Guinea-fowls.*

Weight of Fowl in Kilos.	Dose of Venom per Kilo.	Result.
1.353	0.0015 gram	The bird hardly seemed to suffer at all, and was certainly quite well a few days later, except for a threatening of sloughing at the seat of injection.
1.289	0.0020 ,,	Was very sick on the third day, with paralysis of the legs and wings; died 80 hours after the injection; wound sloughing at time of death.

NOTE.—Though the series is very incomplete, it seems clear that these birds do not present any well-marked resistance to the venom of the cobra. A common fowl to which I gave 0.003 gram of cobra venom died in six hours and a half. Captain Gabbett has kindly undertaken to work this matter out more in detail later on.

TABLE G.—*Two Experiments with Peafowl, designed to Ascertain whether these Birds are to any extent Protected against the Venom of the Cobra.*

Weight of Fowl in Kilos.	Dose of Venom per Kilo.	Result.
2.0757	0.0025 gram	Died in 5 hours.
2.1400	0.0035 ,,	Died in 2 hours.

These experiments were kindly performed for me by Captain Gabbett, I.M.S., of Salem, with a carefully-weighed specimen of venom sent him by me. Captain Gabbett calls attention to the fact that the usual method of weighing a fowl with its feathers is unfair to the peafowl, owing to the fact that the relatively large amount of plumage it possesses increases its apparent weight, and therefore raises its dose of venom.

he was unable to work the matter out. A reference to Table D will show that the idea is not altogether devoid of foundation, for although a dose of 0.001 gram per kilo of body weight proved fatal to both pigs and dogs, whilst 0.00075 gram per kilo failed to kill either animal, yet the duration of life was distinctly greater in the pig. The same evidence of increased resistance may be traced where higher doses of venom were given. I incline therefore to the view that the pig possesses a slightly greater degree of resistance to venom than the dog does, even when the dose is graded according to kilos of body weight, and to this one must add that the former animal is

much larger and possesses a very much tougher skin; indeed, it is a difficult matter to force a hypodermic needle through a pig's skin, even after selecting its softest spot.

Before closing this subject I would invite attention to Tables E and F. From the former we learn that the guinea-fowl possesses a slightly greater degree of protection than the common fowl. Calmette has explained the resistance of the latter to venom as being due to its reserve air-sacs.

Comments on Table F will be found in a footnote to it.

Does the Indian Snake Charmer possess any Degree of Immunity from the Effects of the Bites of Poisonous Snakes?

In order to answer the above question, I have been at considerable pains to collect information from a variety of different sources. As the outcome of these inquiries, I have been led to the following conclusions:

1. The snakemen of Southern India trust as a rule to valueless vegetable drugs or mixtures of drugs With but few exceptions, they know absolutely nothing of prophylactic inoculations, or of any other measures beneath which a substratum of scientific reasoning can be traced.

2. A few exceptional charmers, here and there, swallow venom or rub it into their limbs, but without any definite ideas as to why they do so. All that one can elicit by inquiry is the reply "It is a custom," and to the Eastern mind this explanation is all-satisfying. It is possible, but I think very unlikely, that these men conceal their knowledge. On this point the evidence of my assistant, Dr. Pinto, is most valuable, as he knows the caste well, and speaks their language fluently. He expresses himself as convinced that the men he deals with for snakes are really ignorant of any method of protection against snakebite. Captain Giffard, who has devoted a good deal of his consideration to such subjects, gives me the following interesting information in a recent letter: "The head snakeman" of Chingleput, "who died of a cobra bite as he was bringing a snake to me, was well known to eat cobra poison whenever he killed a cobra; he was employed killing snakes, was supposed to be invulnerable, and had a reputation for snakebite curing, by means of medicines administered to his patients by the mouth (some sort of vegetable medicines). The other men hereabout do not eat poison, but rub it on their arms."

3. A surprisingly large number of snakemen appear to lose their lives in the pursuit of their vocation. Within the last year Captain Giffard and Dr. Pinto have each reported a death among the men they employ to assist them in collecting venom with a view to help me in my experiment. It would be easy to multiply instances, but space does not permit this being done; suffice it to say that a number of the fatal cases among these men occur while the victim is under the influence of liquor, and that no room can be left for doubt that the South Indian snake charmer is not highly immunised against venom. On the other hand, it is but fair to quote an extract from a letter received by the writer from Major Lloyd, of the 19th Madras Infantry, a gentleman who spends much of his spare time in studying the ways of venomous snakes. He writes: "Colonel Watson and I saw a snake charmer bitten by a cobra in Behrampore. The cobra was a fresh one he had recently caught, and its poison bag was intact; he was bitten on the forefinger knuckle, where it joins the hand, and I saw

blood on the wound.......The man laughed, and at once produced several pills, of which he swallowed two, rubbed in another over the bite, and went on with his performance." Major Lloyd describes how he kept a close watch on the cobra, and as soon as the performance was over made the charmer close the snake's jaws on a fowl's leg, with fatal results to the bird in ten minutes. It seems possible that this man had succeeded in attaining some measure of immunity.. I have met with snakemen who showed me scars which they asserted were the results of bites by cobras; there was no proof forthcoming as to the correctness of their statements.

4. Many of the men who deal in poisonous snakes undoubtedly take early means to secure themselves against a bite by breaking off the poison fangs. There are two well recognised means of doing this: one is to seize the snake close behind the head, and force open the jaws with a bit of stick, which is then passed behind the fangs and drawn forcibly forwards; the other is to throw a piece of rag tied on to a string to the cobra, and when it bites to twitch the rag with the impaled teeth out of the animal's mouth with a jerk. Lieutenant-Colonel O'Hara, I.M.S., tells me of a third protective method—namely, the production of an artificial internal fistula by tapping the poison sac through the mouth and far back with a sharp instrument. I have no experience of this practice. Professor Calmette, in his most interesting work *Le Venin des Serpents*, commits himself to a statement which lies outside my experience, when he says on p. 48, "Certains jongleurs exhibent des serpents auxquels ils ont pris soin d'arracher les croquets ou *d'extirper les glandes*......" The italics are my own, and refer to the statement to which I take exception; speaking from a large experience of Indian snakemen and their ways, I have never met with a case in which there was any evidence that the glands had been extirpated, nor do I believe that these ignorant men would be capable of performing such an operation. On the other hand, one can unhesitatingly endorse Professor Calmette's assertion that many of the snakemen carry on their performances with cobras whose venom apparatus is intact. This leads us naturally to our next conclusion, which we cannot do better than put in Professor Calmette's own words, which deserve a hearty endorsement.

5. "C'est grâce à une connaissance parfaite des habitudes et des mouvements du reptile qu'ils (the snakemen) évitent presque toujours d'être mordus." This was the conclusion I had arrived at before I had the pleasure of seeing Dr. Calmette's paper, but I cannot go so far as to say with the French observer: "Il ne paraît donc pas qu'ils sachent se donner l'immunité contre le venin par un procédé quelconque." The comparative immunity amongst snake charmers is undoubtedly due to the fact that these men understand the cobra well, and are, therefore, seldom bitten. You will never see a snakeman make a sudden movement when dealing with a cobra; every action is slow and deliberate, and often pervaded with a sense of rhythm; there is a consciousness of power over the reptile that appears to mesmerise it. I have seen a charmer sit on the ground in the characteristic position of the East, within easy striking distance of a cobra, and, without touching the animal, mesmerise it till it followed mechanically every movement of his swaying knee the while that the man's attention was drawn elsewhere.

The photographs on pages 3 and 4, taken by the writer and unfortunately damaged by climate, illustrate, better than any words can, to one who has not watched a charmer at work the extraordinary power these men exercise. It will be observed that in both cases the man's eyes have left the cobras and are fixed in rapt attention on the camera.

A gentleman who handles poisonous snakes freely, and who does not hesitate to thrust his naked hand into a box containing a number of cobras, gives this advice to those who wish to follow his hazardous example: "Never be afraid of the snakes, never make a sudden move, and you are quite safe." It is, however, quite a different matter when one comes to deal with Russel vipers; they are sluggish, uncertain-tempered snakes, which do not rise to strike but spring out from their coil with a suddenness and rapidity which are appalling. I had a very narrow escape whilst experimenting some years ago with a Russel viper, with a view to ascertain its maximum height of strike; the viper, which was lying on the ground, suddenly shot out, like the crack of a whip, aiming its blow not at the guinea-pig tied to a stick which I was offering it as a bait, but at my leg. Now remembering the character of this viper, it is noteworthy that one never sees the ordinary snake charmer with one in his possession; indeed one has the greatest difficulty in persuading snakemen to catch and bring specimens of the daboia; they know well that their blandishments are thrown away on this snake, charm they never so wisely.

We may sum up our conclusions in a very few words:

1. The snakemen of South India are certainly ignorant of any method of producing in themselves a highly-developed condition of immunity.

2. Some few of them appear to practise the swallowing of venom, or the inunction of venom into their limbs, but it is doubtful if they do so with any well-defined object. It is possible that they thus obtain some degree of immunisation.

3. They confine themselves almost exclusively to the cobra, and escape harm by their intimate knowledge of the methods of handling this snake.

It only remains for me to express my sense of indebtedness to the many who have helped me in this work. Though they are too numerous to mention individually, there are three names I must not omit, namely, those of Captain Giffard and Captain Gabbett, of the I.M.S., and of Mr. Charles Gray of Coonoor.

REFERENCE.
1 *Vide* Lydekker's *Royal Natural History*.

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ON THE VALUE OF THE SERUMS OF THE RUSSEL VIPER AND THE COBRA, AS ANTIDOTES TO THE VENOMS OF THOSE SNAKES.

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I feel that some apology is due for the publication of a paper which, at the best, is only a fragment, but it seems a pity to throw away the work of months, because one has been unable to bring it to that completeness one would have desired. Circumstances over which I have no control, prevent me from continuing this line of investigation for the present, and it may be long before the opportunity to take up the thread again presents itself. I have, therefore, decided to avoid theoretical questions, as far as possible, and to limit myself to a publication of the tables with short comments on each.

The original scheme for these experiments contemplated an estimation of the antidotal power of the serums of venomous snakes as exhibited against the venom of the snake from which

the serum was derived, followed by an estimation of the value of the serum of one poisonous snake for the venom of another species; it was then proposed to work out the value of the serums of non-poisonous snakes and of mongooses.

Unfortunately only a small part of the programme has been carried out up to the present.

Of the many imperfections of the work, I am all too conscious; if, however, the lenient reader should by chance find therein aught that seems of suggestive value, I would ask him to credit that to Edinburgh's distinguished pioneer in the field of snake venom research, whose address delivered at the Royal Institution of Great Britain on March 20th, 1896, first suggested the lines on which this research has been conducted.

Method Employed to Separate Serum from Snake's Blood.

With the above end in view, a number of different methods have been tried, and of them all, the following has been found to work best:—

The snake is rendered unconscious by placing it in a box, into which pledgets of cottonwool soaked in chloroform are dropped through an aperture, specially made for the purpose; from time to time the box is shaken, and when all active movements inside have ceased, the lid is raised, and the animal's neck is seized close behind the head with a pair of strong forceps, about 2 ft. long; with these forceps the reptile can be quickly and safely removed to the operating table, provided that care is taken to get a good hold of the spinal column and not to be satisfied with a skin-grip; the neglect of this precaution in one of the earlier experiments

nearly involved serious consequences; a good and convenient form of operating table is a large deal box, standing about 32 inches in height. On to one of the vertical sides of this box, the snake's head is nailed, ventral surface upwards, at such a level that the junction of the head and neck corresponds to the top of the box, the snake's body lying on the upper surface of the box, and the tail being entrusted to an assistant to hold. It is important to use a long nail for the head, as during the process of nailing down, the snake erects its fangs, and expels poison. Owing to the want of this precaution the writer received, what might have proved a very serious wound on one occasion, his thumb slipping down from the nail and becoming impaled deeply on one of the fangs. In order to eliminate this source of danger, a nail 6 inches long, and brought to a fine point at the end is now used for fixing the head; the fangs are next nipped off with bone forceps and removed.

The reptile lying ventral surface upwards, a median vertical incision, two inches long, is made, terminating half inch above the angle of the lower jaw, the skin flaps are carefully dissected back, and held outwards in the grasp of sterilised catch forceps; the left cervical vein, which is constantly the larger, is now dissected up from all its connections for a length of an inch or more, a pair of pressure forceps are applied on the distal side, as close to the head as possible, and the vein is cut clean across with sharp scissors just on the central side of the forceps; the jet of blood is directed with the aid of the forceps into a sterilised glass vessel kept ready for the purpose. The assistant in charge of the tail now raises it, and with it the body into a

nearly vertical position, thus rendering the nailed-down head the lowest part of the animal.

The blood readily runs out and fills the tube, and so thoroughly does this process drain the body, that it is but seldom any advantage to cut across the left vein, or to open the body and repeat the manœuvre on any of the other veins of the animal.

It is most important not in any way to disturb the blood once drawn, and it is, therefore, advisable not to mix the blood from different animals, but to keep each in a separate tube, otherwise a clear serum is not obtained, but one stained with hæmoglobin.

Each tube is next covered with several folds of cloth, which has been boiled in 1-20 carbolic lotion, and is put away in the dark for 48 hours, at the end of which time the serum has been separated, and can be decanted off into sterilised tubes belonging to a centrifugal machine.

Any remnants of fibrin floating in the serum are soon driven to the bottom of the tubes by brisk centrifugalisation, and the liquid is ready for injection. It is hardly necessary to state that every manipulation is carried out with strict attention to the maintenance of antisepsis.

Methods of Exhibiting the Serum.

These may be divided into three main classes :—

(*a*). Those in which the venom was mixed with the serum in vitro, and the mixture injected.

(*b*). Those in which the serum was injected first, and later, after an a interval, the venom followed.

(*c*). Those in which the venom was first given, and then later on the serum followed.

It will be at once observed that while method (*c*) is the one which most nearly approximates to our method in the exhibition of an antidote in cases of snake-bite, the other two methods will more readily show the influence of feeble antidotal powers should these exist.

Again while method (*a*) invites success, if the antidotalism sought for is presumed to be a chemical one, we should expect the results to be in favour of method (*b*) on the presumption of a physiological antidotalism existing.

All these methods have been tried as will be seen.

In most of the experiments the serum has been used subcutaneously, but the intravenous mode of administration has also been given a trial. Of the latter method I shall have nothing to say at present, as my results with it have been very unsatisfactory, doubtless owing to errors which I shall in time learn and avoid.

Daboia Venom and Serum.

Brief notes on Table A.—The first five experiments in this series are instructive. They point strongly towards the conclusion that the serum of the Daboia, when mixed with the poison of that snake, exercises a distinct antidotal action. It will be observed that the dose of venom chosen was sufficient to cause the death of a rabbit in under 14 hours; against this lethal dose, 1 c.c. per kilo of serum proved of no avail, 3 c.c. enabled the animal to live 24 hours, 5 c.c. prolonged life three days beyond this period, and 7 c.c. seemed as if it were going to save the subject, which died, however, on the eighth day.

The consistency of these results throughout the series is worthy of notes.

The next four experiments are equally instructive. It will be noted that with the raised dose of venom, the control succumbed in $4\frac{1}{4}$ hours, while the animals treated with the antidote lived much longer periods, the rabbit which received 3 c.c. living $16\frac{1}{4}$ hours, that which received 5 c.c. living 36 hours, and that which received 7 c.c. living $26\frac{1}{2}$ hours.

In experiments 10 and 11 we find the animal which received 2·5 c.c. surviving altogether, a dose similar to that which the animals 1—5 received.

No reasonable doubt is here left that the influence of the serum was distinctly, though by no means powerfully, antidotal, under the conditions of administration after admixture.

Notes on Table B.—The first six experiments of this series speak for themselves. The serum used was an excellent sample, and the brief notes recorded show the most decided increase of antidotal effects as the dose of serum was raised.

Attention is also drawn to the note which precedes the last two experiments of the series. I would not have ventured to make the observation there recorded, had it not been the outcome of indications frequently repeated. It may be asked, why under the circumstances one ever uses stained serum; the answer is a simple one, *viz.*, that an absolutely clear specimen of snake serum is difficult to obtain, even when the greatest care is taken. Let anyone who doubts this try for himself.

Notes on Tables C and D.—The value of Table C is very seriously diminished by the fact that the series was unfortunately uncontrolled. The

consistency of the results as evidenced by the order in which the animals died, and by the survival of those with large doses of the remedy, leads me to publish the series in spite of the drawback above-mentioned.

In comparing these results with those in Table A, it is to be remembered that fowls are much more resistant to the action of venom than rabbits are. With these remarks the table may be left to speak for itself.

Table D is very suggestive. On the one hand, it indicates decided, though feeble, protection on the part of the serum when administered subsequently to the injection of the venom, and, on the other hand, it suggests that large doses of serum act less advantageously than small ones. I offer no explanation.

An allusion has been made in the notes on Table B to the fact that when using blood stained serum, I have noticed that though the antidotal power of the fluid is not definitely destroyed, there seems to be added an element of toxicity from the serum itself. This observation has been so often made by me that it seems to call for some explanation. I offer one tentatively only, and with much diffidence, for I feel that the work is far too incomplete for dogmatism. It seems possible, either that the blood pigments of serpents' blood are themselvès poisonous, or else that there escapes into the blood along with the pigment some noxious substance or substances. The relation of such substances to the antidotal constituent or constituents of the serum I am not prepared at present to discuss.

The interest of the above observations centres in the fact that while Professor Calmette declared

the blood of poisonous snakes to be itself poisonous, Professor Fraser was the first to discover that the serum of these serpents had distinct antidotal powers.

Several other series were undertaken on the same lines as the experiments shown in Tables C and D, but as the results of these were for one reason or another inconclusive, it does not seem worth while to publish them.

Finally a comparison between Tables B and C is worth making. The advantage of administering the serum before the remedy is here indicated in the fact that whereas 3 c.c. of serum failed to save the fowl, which had received its dose of venom half an hour beforehand, the same dose proved protective when given two and a half hours before the poison. The notes record that the fowl showed but little sickness, and that only for two or three days.

A still more striking proof is found when we come to compare the fowls which received only 2 c.c. of serum each. Here we find that the bird in whose case the poison was given the start, died in less than 16 hours, while that in which the remedy was given beforehand recovered, though decidedly sick for three days.

It is to be greatly regretted that Tables A and B do not admit of similar comparisons; the change of subjects from rabbits to fowls was forced on me by the supply of the former animals running short.

Notes on Tables E and F.—In view of what has been written in the notes on Daboia serum, it would be superfluous to write much here. The tables are published, and inasmuch as they support the results obtained with Viperine serum, they may be considered to be not without interest.

The first series of Table E, consisting of six experiments, is suggestive owing to the consistency of the results, the uniformity of protection being only broken by experiment four; this slight deviation may be due to the idiosyncrasy of the animal, or possibly the rabbit may have been unwell, and this fact has been in some way overlooked.

Experiment seven shows a measure of protection with the anticipatory administration of 4 c.c. of serum.

Table F is very fragmentary, but it is not devoid of suggestive interest.

General Deductions.—It seems clear that the serum obtained from the blood of venomous snakes is, when obtained pure, antidotal in its properties. The antidotal power possessed does not, however, appear to be of a sufficiently high order to warrant the hope that the blood of these dangerous reptiles will yield a fluid of commercial value as an antidote to snake-bite, unless means are found of separating the antidotal constituent from the serum. More than this I am not at present prepared to say.

In closing this paper, I would tender my thanks to the many medical men and laymen who have given me much useful and ready help.

Table A.

Experiments designed to ascertain whether Daboia serum (fresh) is an antidote to Daboia poison, when the two are mixed and injected subcutaneously.

The strength of the solution of venom used was ·001 grm. per c.c. of freshly boiled, cooled water; the solution of venom was mixed with the dose of serum before injection, the two were

drawn up into the syringe and injected into the flank of the rabbit used.

	Dec. '99.	Wt. in kilos.	Dose per kilo.	REMARKS.
1	18th	·61627	·003 grm. venom	Control; died in between 6 and 14 hours; found dead in the morning.
2	18th	·58796	·003 grm venom. 1 c.c. serum.	Died in between 6 and 14 hours; found dead next morning.
3	18th	·58089	003 grm. venom. 3 c c. serum.	Died in 24 hours.
4	18th	·61627	·003 grm. venom. 5 c c. serum.	Was sick for a day, then improved, got convulsions on 22nd, and died four days after injection.
5	18th	·6729	·003 grm. venom. 7 c.c. serum.	Was sick for a day, then got better and seemed well on the 5th day after injection. Died on 8th day.
6	18th	·9068	·004 grm. venom.	Control; died in 4 hours 15 minutes.
7	18th	·99878	004 grm venom. 3 c.c.serum.	Died in 16 hours 15 minutes.
8	18th	·98ˢ8	·004 grm. venom. 5 c c.serum.	Died in 36 hours.
9	18th	·8146	004 grm venom. 7 c c. serum.	Died in 2€½ hours.

Another short series similar to the above.

	Dec '99.	Wt. in kilos.	Dose per kilo.	REMARKS.
10	20th	·8076	·003 grm. venom.	Control; died in 13½ hours.
11	20th	·9847	·003 grm. venom. 2·5 c.c. serum.	Recovered.

TABLE B.

Series of experiments on fowls to ascertain whether the previous injection of doses of

Daboia serum, protects an animal against the injection of lethal doses of Daboia venom under the skin.

NOTE.—All these experiments were performed on Dec mber 21st, 1899; the serum was given about two and a half hours' start; it was injected into the muscles and s bcutaneous tissue; the poison was injected in every case into the subcutaneous tissue under the wing, a position which had not been used for the injections of serum. No obstacle to the absorption of the poison could thus have been offered.

	Weight in kilos.	Dose per kilo.	REMARKS.
1	·921	·003 grm. venom.	Control. Died in 21½ hours.
		6c.c serum.	
2	·75092	·003 grm. venom. 5c c serum.	Was never really sick; the wound caused by the injection threatened to slough. Recovered.
3	·94928	003 grm venom. 4c.c. serum	Sick for one or two days. Recovered.
4	·93512	·003 grm. venom. 3c c. serum.	A little sick for 2 or 3 days. Recovered.
5	·82175	·003 grm. venom. 2c.c. serum.	A little sick for 2 or 3 days. Recovered.
6	·86427	·003 grm. venom.	Decidedly sick for 3 days. Recovered.

The serum used for the above fowls was a clear straw colour; a little blood-stained serum was left over, which was used for the next two experiments, and the results strongly suggest that the serum whilst it protected against the venom was in itself nocuous.

	Weight in kilos.	Dose per kilo.	REMARKS.
7	1·0626	3c.c. serum ·003 grm. venom.	Died in 67 hours.
8	1·0413	8c c. serum. ·003 grm. venom.	Very sick from the first; died on the fifth day.

Table C.

Series of experiments in which a fatal dose of Daboia venom having been first administered, Daboia serum was subsequently exhibited subcutaneously, with a view to test the antidotal value of the latter.

The experiments were performed on 24th December 1899, on fowls.

	Hour.	Wt. in kilos.	Dose per kilo.	Remarks.
1	1-2 P.M. 1-32 P.M.	·77215	·003 grm. venom. 2 c.c. serum.	Died in under 16 hours.
2	12-52 P.M. 1-29 P.M.	·786	·003 grm. venom. 3 c.c. serum.	Died in 36 hours.
3	1-5 P.M. 1-35 P.M.	·765	·003 grm. venom 4 c.c. serum.	Recovered.
4	1-7 P.M. 1-37 P.M.	·8075	·003 grm. venom. 5 c.c. serum.	Recovered.
5	1-9 P.M. 1-45 P.M.	·8216	·003 grm. venom. 7·9 c.c. serum.	Recovered.

N.B.—It is to be regretted that this series is uncontrolled, but the results are so consistent throughout that there can be but little doubt that decided protection was afforded by the serum.

Table D.

A series of experiments in which a fatal dose of Daboia poison having been first administered, Daboia serum was subsequently exhibited subcutaneously, with a view to test the antidotal power of the serum.

The experiments were performed on the 18th January 1900, the subjects chosen being fowls, and the venom being given a start of between

34 and 38 minutes in each case. The serum used was stained with blood pigment.

	Hour.	Wt. of fowl in kilos.	Dose per kilo.	Results.
1	12·57 P.M.	1·006	0·004 grm. venom	A control experiment; fowl found dead 16 hours after injection.
2	1-0 P.M 1-34 P.M.	0·963	0·004 grm. venom. 2 c.c. serum.	Died in 9 days.
3	1-1 P.M. 1·35 P.M.	0·907	0·044 grm. venom 3 c.c. serum.	Died on 26th day.
4	1-2 P.M. 1-35 P.M.	0·006	0·004 grm venom. 4 c.c. serum.	Died on 21st day.
5	1-3 P.M. 1-40 P.M.	1·020	0·004 grm. venom. 5 c.c. serum.	Died on 19th day.
6	1-4 P.M. 1-42 P.M.	0·907	0·004 grm. venom. 6 c.c. serum.	Died in 25 hours.

Table E.

Experiments designed to ascertain whether Cobra serum is an antidote to Cobra venom.

The venom was dissolved in freshly boiled cooled water at a strength of ·001 grm. per cc. of water; it was mixed with the dose of serum, and injected into the flank of a rabbit.

	Date Nov '99.	Wt. in kilos.	Dose per kilo.	Remarks.
1	26th	1·33934	·0007 grm. venom	Control; died in 13½ hours.
2	26th	·82214	0007 grm. venom. 1 c.c. cobra serum.	Unwell for 2 days then improved, and died on 14th day.
3	26th	1·3607	·0007 grm. venom. 2 c.c. serum	Followed much the same course as the previous rabbit; died on 16th day.

	Date. Nov. '99	Wt. in kilos.	Dose per kilo.	REMARKS.
4	26th	·85049	·0007 grm. venom. 3 c.c. serum.	Followed course similar to above two rabbits, but died on 7th day.
5	26th	1·47417	·0007 grm. venom. 4 c.c. serum.	As above; died on 24th day.
6	26th	1·07728	·0007 grm. venom. 5 c c. serum.	Was sick for only a day; is alive and well 25 days after injection.

In the following two experiments, the serum was injected in one flank and followed one hour later by an injection of poison in the opposite flank:—

	Date. Nov. '99.	Wt. in kilos.	Dose per kilo.	REMARKS.
7	26th	·992234	4 c.c. serum. ·0007 grm. venom.	This rabbit was very sick for several days, then got better, and eventually died on 12th day.
8	26th	·850492	2 c c. serum. ·0007 grm. venom.	Died in eight hours.

The serum used in the experiments was freshly prepared; it was a clear straw colour.

TABLE I.

Series of experiments on fowls designed to ascertain whether Cobra serum, exhibited previously to the administration of a lethal dose of Cobra venom, proves to have antidotal powers against the poison.

NOTE.—The experiments were performed on March 4th, 1900. The serum was given about one and a half

hours' start in each case; all injections were made into the subcutaneous tissue, care being taken that the venom was not injected into the same area as the serum.

	Hour.	Wt. in kilos.	Dose per kilo.	RESULTS.
1	2-37 P.M.	0 914	0·0025 grm. venom.	Dead in 7 hours.
2	1-6 P.M.	0·708	2 c.c. serum.	
	2-38 P.M.		0·0025 grm. venom.	Found dead 17 hours after the injection.
3	1-9 P.M.	0·8146	3 c.c. serum.	
	2-42 P.M.		0·0025 grm. venom.	Recovered.

The serum used for the above two experiments was a clear good sample; there was some *blood-stained* serum also available, and this was used with the next two animals.

Hour.	Wt in kilos.	Dose per kilo.	RESULTS.
1-11 P.M.	0·793	4 c.c. serum.	
2-44 P.M.	......	0·0025 grm. venom.	Died in 18 hours.
1-16 P.M.	0·822	5 c.c. serum.	
2-45 P.M.	......	0·0025 grm. venom.	Found dead in 17 hours.

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